

# Two major QTLs on chrs. 2BL and 3BS influence grain yield in durum wheat across a broad range of water regimes

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## ABSTRACT

Coping with reduced inputs, especially in terms of yield stability under the various stress conditions that frequently occur in the drought-prone areas of the Mediterranean basin, is a main objective for durum wheat improvement. A total of 249 RILs (Kofa × Svevo) were evaluated in 16 trials conducted in 2004–2005 under a broad range of water input and yield potential. Two major, epistatic QTLs on chrs. 2BL and 3BS influenced grain yield and related physiological traits in a wide range of environments. In both cases, coincidence between the QTLs for grain yield and those for plant height, peduncle length, SPAD, NDVI index and kernel weight was observed. Epistasis favoured the parental genotypes. On a mean basis, the  $R^2$  values for grain yield of the 2BL and 3BS QTLs were equal to 21.5 and 13.8%, respectively. The effects of these two QTLs were validated in a set of 11 trials conducted in 2006 using 40 selected RILs.

## INTRODUCTION

Durum wheat (*Triticum durum* Desf.) is primarily grown under rainfed conditions where the frequent occurrence of drought combined with heat stress are the major factors limiting grain yield (Araus et al., 2002, 2003; Condon et al., 2004). Improving durum wheat for grain yield and yield stability is therefore a major goal for Mediterranean countries growing durum under rainfed conditions.

The identification of QTLs with major and environmentally stable additive effects is even more desirable when targeting drought-prone environments where the spatial and temporal phenotypic variation (including  $G \times E$  effects) is usually larger than that observed in favorable environments (Lanceras et al., 2004), a condition that lowers the heritability of target traits. Under such a contrasting scenario, the identification of major QTLs characterized by a limited  $G \times E$  is highly desirable to enhance productivity and facilitate their cloning (Tuberosa and Salvi, 2006). In this study, a durum wheat recombinant inbred population obtained from two elite cultivars was evaluated during three growing seasons in Mediterranean environments to identify QTLs for grain yield and its determinants.

## MATERIALS AND METHODS

A population of 249 recombinant inbred lines (RILs) was produced by Società Produttori Sementi (Bologna, Italy) from the elite durum cvs. Svevo (S) and Kofa (K). Svevo has been obtained from a CIMMYT line (Bittern/Yavaros79 germplasm) and the cv. Zenit (Valricardo/Vic). Kofa is a Southwestern US cv. released by Western Plant Breeders (Arizona, USA) and obtained from a multi-parental population (dicoccum alpha pop-85 S-1) with founders related to the American, Italian and CIMMYT germplasm. Both Svevo and Kofa are well adapted to the Mediterranean climate and can be classified as early flowering genotypes in such conditions.

Sixteen field trials, considered as environments (envs.), were carried out over 2004 and 2005 in Italy, Spain, Morocco, Tunisia, Syria, and Lebanon, under rainfed and irrigated conditions. RILs were tested in unreplicated field plots (4 m<sup>2</sup>) adopting a modified augmented design with three checks (Kofa, Svevo and Vitron). The following traits were considered: heading date (HD), plant height (PH), peduncle length (Pd), grain yield (GY) and its components such as thousand kernel weight (TKW), number of kernels per m<sup>2</sup> (Kpsm), number of fertile tillers per m<sup>2</sup> (FTpsm), mean number of grains per m<sup>2</sup> (Kpsm), and grain volume weight or test weight (TWT). Among physiological traits, flag leaf greenness (SPAD chlorophyll meter, Minolta) and the NDVI spectroradiometrical index (normalized difference vegetative index, GreenSeeker<sup>TM</sup>) were taken at mid grain filling.

A genetic map based on 232 SSRs with 23 linkage groups, covering in total 2,347 cM (Haldane's mapping function), and an average marker distance of 10.2 cM, has been assembled and used for QTL analysis. Phenotypic data were analyzed by Restricted Maximum Likelihood (REML) to fit a mixed model with checks as a fixed effect and rows, columns, and unreplicated entries as random effects. REML model produced Best Linear Unbiased Predictors (BLUPs) for the phenotypic data used in QTL analysis. QTL and QTL×QTL epistatic interaction analyses were carried out using Composite interval mapping (CIM) and multiple interval mapping (MIM) analyses in WinQTL Cart v. 2.5 as described in Maccaferri et al. (2008).

Based on the results obtained from the two years of field testing, 40 RILs were selected based on their haplotypes at the two major QTL clusters for yield and related traits

located on the 2BL and 3BS distal chr. regions. Composition of the 40 selected RILs at the two QTLs was as follows: 10 RILs: + + / + +, 10 RILs: + + / - -, 10 RILs: - - / + +; 10 RILs: - - / - -. The 40 selected RILs were evaluated in 2006 in 11 replicated field experiments (3 reps) with plots of 6 m<sup>2</sup>.

## RESULTS AND DISCUSSION

### *Variation among environments and RILs*

The genetic materials were tested under a range of photothermal conditions and water availabilities, representative of the variation present in Mediterranean climates. Crop cycle length (emergence to harvest) ranged from 135 days (Lebanon, 2004, rainfed conditions) to 213 days (Syria, 2004, irrigated conditions); heading to harvest period (significantly correlated with the final GY) ranged from a minimum of ca. 40-50 days (Lebanon, Tunisia, Morocco) to a maximum of ca. 80-100 days (Syria and Italy). Water inputs during the crop cycle ranged from ca. 50 to ca. 350-400 mm. Based on the mean GY of the RILs, five envs. were considered as highly productive (mean GY > 50 q ha<sup>-1</sup>), six as medium yielding (GY from 25 to 50 q ha<sup>-1</sup>) and four as low yielding (with GY < 25 q ha<sup>-1</sup>, all under rainfed environments). PH was positively correlated with GY in 13 out of 16 envs. HD was significantly correlated with GY in only six envs. with *r* values consistently negative. The two parents showed a high and similar productivity level, as expected for elite, early heading cultivars adapted to Southern Mediterranean envs. Their average performances were similar to those observed for cv. Vitron, a cv. with high and stable yield potential in the Mediterranean areas. Heritability values across envs. were high for HD, PH, Pd and TKW (0.80-0.95), medium for GY, TW, Kpsm and SPAD (from 0.40 to 0.67), low for FTpsm and NDVI (less than 0.40).

### *QTL analysis results*

Considering each env. separately, the phenotypic variation accounted for by all QTLs ranged from ca. 10 to 40% for GY and Kpsm and from ca. 20 to 55% for TKW, HD, and PH, including the epistatic interaction effects among additive QTLs. For each trait, the majority of the QTLs were found to be specific for a single environment, where they frequently showed appreciable *R*<sup>2</sup> values. The highest number of QTLs was detected for Kpsm and for GY (21 and 16 QTLs in total, respectively), with 14 environment-specific QTLs for GY and 13 for Kpsm. A few of these unique QTLs showed *R*<sup>2</sup> values that were quite high: from 4.0 to 13.5% for GY and from 2.5 to 17.0% for Kpsm.

Among the QTLs that were detected across environments, two main QTLs, one on chr. 2BL and the other on chr. 3BS, both in the distal chr. regions, concomitantly influenced GY and several related traits over a total of eight and seven envs., respectively. Each of these two QTLs was also found to have a significant effect on PH, Pd, yield components (particularly TKW), TW, SPAD and NDVI across a range of environments.

LOD peak positions across environments varied within a distance range equal or inferior to 20 cM. These two QTLs were referred to as two major “QTL-clusters” and indicated as *QYld.idw-2B* and *QYld.idw-3B*. SSR markers flanking *QYld.idw-2B* were: *Xgwm846*, *Xgwm1027*, *Xwmc361* (located within a 10 cM interval), while for *QYld.idw-3B*, flanking markers were: *Xgwm1034*, *Xbarc133* and *Xgwm493* (within a 11.1 cM interval). The additive favourable effects were contributed by Svevo at *QYld.idw-2B* and by Kofa at *QYld.idw-3B*.

Even if the two QTL clusters were detectable over a range of environments with different yield capability, they could not be detected under heavily stressed environments. An interesting feature of these two QTL-clusters is that both the two chromosome regions did not show noticeable constitutive effects on heading date. Major QTLs for heading date were found on chromosome 2AS, 2BS, 2BL and 7BS, but they showed only minor QTL effects on GY.

The QTL clusters were also detected for most traits using the mean values across the 16 envs. Considering the GY mean values, the 2BL and 3BS QTL regions showed LOD peak values of 8.9 and 10.0, *R*<sup>2</sup> values of 15.6 and 15.3% and a similar additive effect of 0.55 q ha<sup>-1</sup> each (CIM analysis). MIM-based QTL analysis confirmed the QTL peak positions of the CIM analysis, and revealed a strong, significant epistatic interaction between the two QTLs.

In the MIM analysis, the two QTL-clusters showed significant epistatic interactions also for the majority of the traits and envs. Epistatic interactions showed a magnitude of *R*<sup>2</sup> values and effects that were comparable to those estimated for the additive QTL effects. The epistatic interaction was positive (favourable) for the two parental (non recombinant) genotypic classes (KK<sub>2BL</sub>KK<sub>3BS</sub> and SS<sub>2BL</sub>SS<sub>3BS</sub>) and negative for the two recombinant classes (KK<sub>2BL</sub>SS<sub>3BS</sub> and SS<sub>2BL</sub>KK<sub>3BS</sub>). This is exemplified in Figure 1 that shows the additive and additive × additive effects on GY and PH in a two marker model. A main consequence of this model is that the genotypic class predicted to reach the maximum GY value based on the additive effect only (SS<sub>2BL</sub>KK<sub>3BS</sub>) failed to maximize GY; moreover, GY was further reduced in the KK<sub>2BL</sub>SS<sub>3BS</sub> genotypes.

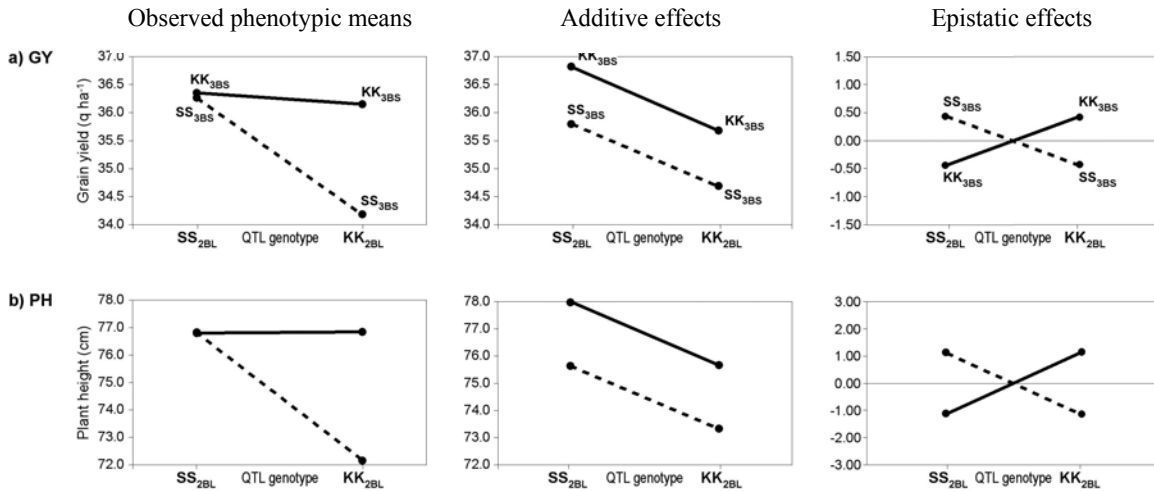
The 11 replicated field trials carried out in 2006 to evaluate the 40 selected RILs confirmed the previous QTL observations (i.e. magnitude and direction of the effects). From six to 20 RILs out of the 40 selected RILs yielded more than the average yield of the five checks. The combined ANOVA results confirmed the presence of highly significant effects at both QTL regions and a highly significant *Xgwm1027* × *Xbarc133* interaction for GY, PH, Pd, TKW, Kps, TW and also HD, but not SPAD and NDVI. Highly significant RILs × envs. and markers × envs. interactions were observed for several traits.

Fine mapping of *QYld.idw-3B* has been undertaken and near isogenic materials are going to be developed via heterogeneous inbred family approach (Tuinstra et al, 1997) starting from four different RIL backgrounds

(chosen among those where the epistatic interaction effect did not mask the phenotypic effect of the allelic

substitution at the target region).

Figure 1. Additive and epistatic effects for grain yield (GY) and plant height (PH) calculated on the data averaged across the 16 environments tested in 2004 and 2005. Results from a two-marker model including *Xgwm1027* for *QYld.idw-2B* and *Xbarc133* for *QYld.idw-3B*.



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